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# Plasma Steroid Hormone Profiles in HCG-Injected Male Walking Catfish *Clarias batrachus*

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**ABSTRACT**—The present experiment was performed in order to investigate the response of matured male walking catfish to human chorionic gonadotropin (HCG).

*Short-term experiment.* Mature male walking catfish received a single intramuscular injection of HCG (0.2 and 1 IU/g BW) or saline. Blood samples were taken at 0, 6, 12, 18, 24, 36, 48 and 72 hr. *Long-term experiment.* Mature male walking catfish received a single intramuscular injection of HCG (1 IU/g BW) or saline. Blood samples were taken at 2-day intervals until day 10. In both experiments, plasma testosterone, 11-ketotestosterone (11-KT), 17$\alpha$-hydroxyprogesterone, 17$\alpha$,20$\alpha$-dihydroxy-4-pregnen-3-one and 17$\alpha$,20$\beta$-dihydroxy-4-pregnen-3-one levels were measured by radioimmunoassay, and 17$\alpha$,20$\beta$,21-trihydroxy-4-pregnen-3-one levels were measured by enzyme immunoassay.

Results can be summarized as follows. 1) HCG firstly induces an increase in plasma testosterone and 11-KT levels. 2) Thereafter, 11-KT levels decrease and progestin levels increase. 3) Finally, progestin levels decrease, and 11-KT levels increase again. These results clearly indicate that a shift in the steroidogenic pathway is induced in males in response to HCG.

## INTRODUCTION

Walking catfish (*Clarias batrachus*) is a freshwater teleost which is widely distributed throughout Southeast Asia. Although this fish has been commonly cultured for a long period, little information is available on its reproductive physiology, especially regarding males. The reproductive organ of the male tropical walking catfish differs from other teleosts such as cyprinids and salmonids. Walking catfish testes are very small; therefore, milt cannot be obtained by applying pressure to the abdomen in the manner that is commonly employed with other fishes. Male walking catfish reared under 23–25°C start to mature at an age of 9 months, and thereafter maintain conditions of maturity. Plasma testosterone and 11-ketotestosterone (11-KT) in males were always maintained at high levels throughout the year under warm water conditions, but progestin levels were low [26].

It has been proposed that during spermiation and spawning, a shift in the steroidogenic pathway from androgen to progestin production occurs in response to increased gonadotropin secretion in salmonids [6, 12, 24], in cyprinids [5], and in a perciform [9]. Progestins, such as 17$\alpha$,20$\beta$-dihydroxy-4-pregnen-3-one (17$\alpha$,20$\beta$-P), and 17$\alpha$,20$\alpha$-dihydroxy-4-pregnen-3-one (17$\alpha$,20$\alpha$-P), have been reported to exist in males, and to increase during spermiation and spawning in response to increased gonadotropin secretion. In addition, another progestin, 17$\alpha$,20$\beta$,21-trihydroxy-4-pregnen-3-one (20$\beta$-S) has been reported to increase in the female Atlantic croacker [23], in the spotted seatrout [22] and in the walking catfish [25] during final oocyte maturation. However, the presence of 20$\beta$-S in male teleosts has not been reported yet.

There still remains a paucity of information on hormonal changes during natural spawning in the male walking catfish; it is generally difficult to follow hormonal changes in individual fish due to

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extreme sensitivity to handling stress. Therefore, in this investigation changes in plasma levels of testosterone, 11-KT, 17$\alpha$-hydroxyprogesterone (17$\alpha$-P), 17$\alpha$,20$\alpha$-P, 17$\alpha$,20$\beta$-P and 20$\beta$-S were monitored in the HCG-treated male walking catfish.

## MATERIALS AND METHODS

*Fish* Walking catfish fingerlings were originally obtained from The Faculty of Fisheries, Bogor Agricultural University, Bogor, Indonesia in 1988. These fish were reared at 23–25°C under 12L12D photoperiod until reaching maturity. Fish began to mature at an age of 9 months and conditions of maturity were maintained thereafter; but under the above stocking conditions, fish were observed not to undergo natural spawning [26].

*Short-term experiment* Twenty-four 2-year old matured male walking catfish (400–650 g in weight and 35–40 cm in total length) were selected from the stock, and then randomly assigned to three groups, injected with 0.2 IU HCG/g BW, 1 IU HCG/g BW and saline. Each group was kept separately in a $1\times3\times1$ m indoor concrete tank supplied with running fresh water of deep-well origin. Water temperature and photoperiod were maintained at $24\pm0.5$°C, and 12L12D, respectively. Fish did not receive feed during the experiment.

*Long-term experiment.* Fourteen 16-month old matured male walking catfish (200–275 g in weight and 30–33 cm in total length) were divided into two groups. Eight fish received 1 IU HCG/g BW, whereas the remaining six fish received saline solution. Each group was kept separately in the same manner as in the short term monitoring experiment.

*Treatment* HCG was purchased from Teikoku Zoki Pharmaceutical Company, Japan. In order to meet the required dosage, the original hormone was diluted with 0.6% saline solution into 0.2 IU/$\mu$l and 1 IU/$\mu$l solution.

HCG treated groups received a single intramuscular injection of HCG, whereas the saline group received an equal volume of saline solution. Injection was done just below the beginning of the dorsal fin at 6 a.m.

*Blood sampling* Initial blood samples were taken from all fish before administering hormone solution or saline. Sampling was carried out at 6, 12, 18, 24, 36, 48 and 72 hr in the short-term experiment. In the long term experiment, sampling was carried out 2, 4, 6, 8, and 10 days after HCG injection (at 10 a.m.). Fish were sacrificed for the measurement of gonadosomatic index (GSI) at the termination of the experiments.

Approximately 0.8 ml of blood was drawn from the caudal vasculature with a heparinized syringe fitted with a 24-gauge, 1 inch needle after anesthetizing fish with 600 ppm of 2-phenoxyethanol (Wako, Japan). Blood samples were centrifuged at 3000 rpm, and plasma was stored in 1.5 ml polypropylene tubes at −20°C until analysis by radioimmunoassay (RIA).

*RIA* In this experiment, plasma testosterone, 11-KT, 17$\alpha$-P, 17$\alpha$,20$\alpha$-P and 17$\alpha$,20$\beta$-P were determined by RIA. Details of RIA for each steroid have been described previously [1, 13, 25]. Validation of the assay system for testosterone, 17$\alpha$-P, 17$\alpha$,20$\alpha$-P and 17$\alpha$,20$\beta$-P has also been previously reported [25].

Validation of the 11-KT assay system for walking catfish plasma was carried out in the same manner as has been reported previously [8]. Intraassay coefficients of variation at binding rates around 25%, 50% and 75% were 6.3, 4.0, and 4.5, respectively. Interassay coefficients of variation at binding rates around 25%, 50% and 75% were 18.6, 9.1, and 19.8, respectively.

*EIA* Plasma 20$\beta$-S levels were measured using a specific enzyme immunoassay system (EIA). Validation for this EIA was reported previously [25].

*Statistics* The Student-t test, and the Duncan's multiple range test and the Kruskal-Wallis test were used for statistical analysis.

## RESULTS

*Short-term experiment*

Changes in plasma testosterone levels following HCG injection are presented in Figure 1. Plasma testosterone levels in 0.2 IU HCG-treated group became elevated at 6 hr ($P<0.01$; 0 hr *vs* 6 hr), reaching a peak at 18 hr (207.4 ng/ml), and thereafter remained higher than initial levels (5.2 ng/ml) until the end of the experiment ($P<0.01$; 0 hr *vs* 72 hr). Testosterone levels in 1 IU HCG-treated group showed changes similar to those of the 0.2 IU HCG-treated group. No significant changes occurred in the control group.

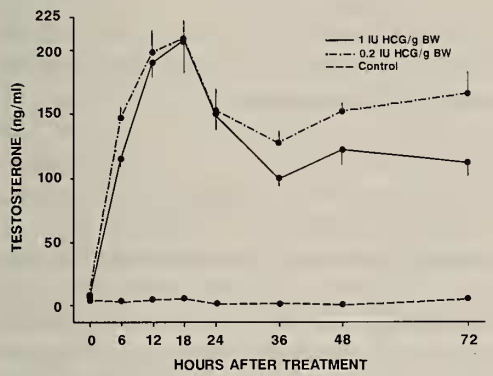


FIG. 1. Plasma testosterone levels of male walking catfish following HCG injection in short-term experiment. Each point represented as mean±SEM (n=8 for each treatment).

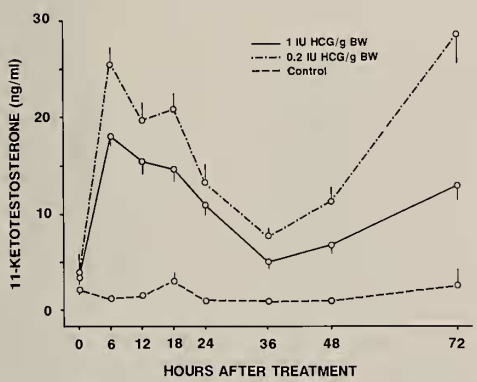


FIG. 2. Plasma 11-KT levels of male walking catfish following HCG injection in short-term experiment. Each point represented as mean±SEM (n=8 for each treatment).

Changes in plasma 11-KT levels are shown in Figure 2. Plasma 11-KT levels in 0.2 IU HCG-treated group peaked at 6 hr (25.6 ng/ml; $P<0.01$; 0 hr *vs* 6 hr), and then decreased until 36 hr (7.6 ng/ml; $P<0.01$, 18 hr *vs* 36 hr), and thereafter increased again until the end of the experiment (28.6 ng/ml). Plasma 11-KT levels in the 1 IU HCG-treated group showed a similar pattern of changes. 11-KT levels in the control group were maintained at low levels during the experiment.

Changes in plasma 17$\alpha$-P levels are presented in Figure 3. Plasma 17$\alpha$-P levels increased from 36 hr until 72 hr in both 0.2 and 1 IU HCG injected groups ($P<0.01$; 24 hr *vs* 72 hr). Levels at 72 hr for 0.2 and 1 IU HCG/g BW were 13.6 ng/ml and 26.2 ng/ml, respectively. On the other hand, no significant changes were observed in the control group.

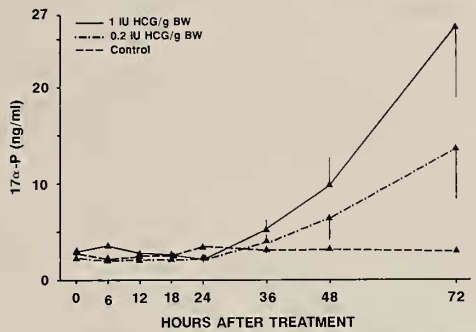


FIG. 3. Plasma 17$\alpha$-P of male walking catfish following HCG injection in short-term experiment. Each point represented as mean±SEM (n=8 for each treatment).

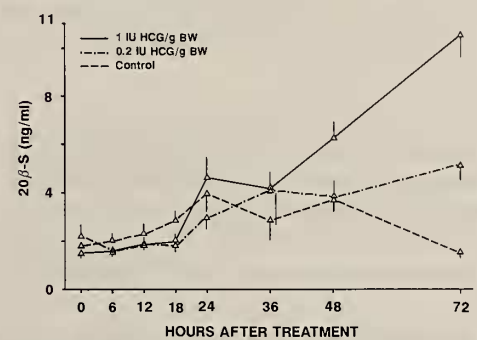


FIG. 4. Plasma 20$\beta$-S levels of male walking catfish following HCG injection in short-term experiment. Each point represented as mean±SEM (n=8 for each treatment).

Changes in plasma 20$\beta$-S levels are presented in Figure 4. Plasma 20$\beta$-S levels in the 1 IU HCG/g BW-treated group started to increase at 24 hr ($P<$ 0.01; 18 hr *vs* 24 hr), and reached 10.5 ng/ml at 72 hr. Plasma 20$\beta$-S levels in the 0.2 IU HCG/g BW-treated group increased in the same manner as those of 1 IU HCG/g BW group, but levels for 48 and 72 hr were lower. Plasma 20$\beta$-S levels in the control group slightly increased during 24–48 hr ($P<0.01$; 0 hr *vs* 24 hr), and then decreased ($P<0.01$; 48 hr *vs* 72 hr).

Changes in plasma 17$\alpha$,20$\alpha$-P levels are presented in Figure 5. Injection of 1 or 0.2 IU HCG/g BW caused a parallel increase in 17$\alpha$,20$\alpha$-P levels, starting from 6 hr ($P<0.01$; 0 hr vs 6 hr) continuing until 72 hr (levels for 0.2 and 1 IU HCG/g BW treated groups were 1.7 and 3.0 ng/ml, respectively). Levels in the control group were always under the detectable limit throughout the experiment.

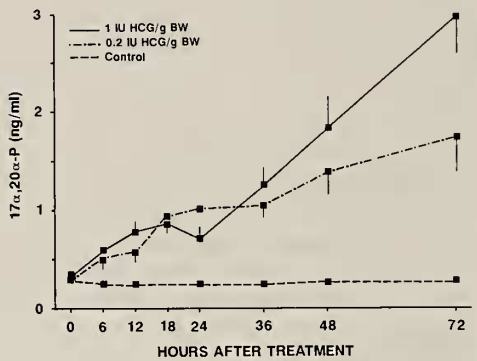


FIG. 5. Plasma 17$\alpha$,20$\alpha$-P of male walking catfish following HCG injection in short-term experiment. Each point represented as mean$\pm$SEM (n=8 for each treatment).

Changes in plasma 17$\alpha$,20$\beta$-P levels are presented in Figure 6. Injection of 1 and 0.2 IU HCG/g BW caused a similar increase ($P<0.01$; 6 hr *vs* 72 hr) in 17$\alpha$,20$\beta$-P levels, starting from 6 hr. This trend continued until 72 hr (2.8 and 4.6 ng/ml for 0.2 and 1 IU HCG/g BW, respectively). Except for values at 24 and 36 hr, plasma levels were higher in the 1 IU HCG/g BW treated group than in the 0.2 IU treated group. Levels in the control group were always under the detectable limit.

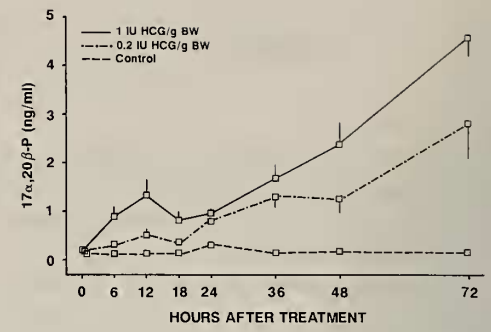


FIG. 6. Plasma 17$\alpha$,20$\beta$-P levels of male walking catfish following HCG injection in short-term experiment. Each point represented as mean$\pm$SEM (n=8 for each treatment).

GSI values at the end of the short-term experiment are presented in Figure 7a. GSI increased significantly from 0.15 % to 0.22% in 0.2 IU HCG/g BW treated group, and to 0.20% in 1 IU HCG/g BW treated group.

*Long-term experiment*

Changes in plasma testosterone, 11-KT, and 17$\alpha$-P levels are presented in Figure 8.

Plasma testosterone levels increased sharply from 9.9 ng/ml to 77.6 ng/ml on day 2 ($P<0.01$; day 0 vs day 2), and then decreased but remained high until day 10 (34.1 ng/ml). Testosterone levels on day 10 were still significantly higher than initial and control levels. No significant changes occurred in the control group.

Plasma 11-KT levels decreased from 5.7 ng/ml

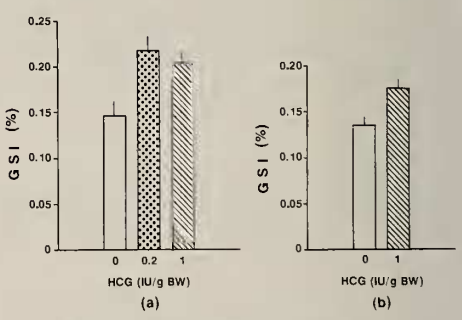


FIG. 7. Changes in GSI values following HCG injection in short-term (a) and long-term (b) experiments. Each bar represented as mean$\pm$SEM (n=8 for each treatment in short-term experiment, and n=8 and 6 for HCG and control group, respectively in long-term experiment).

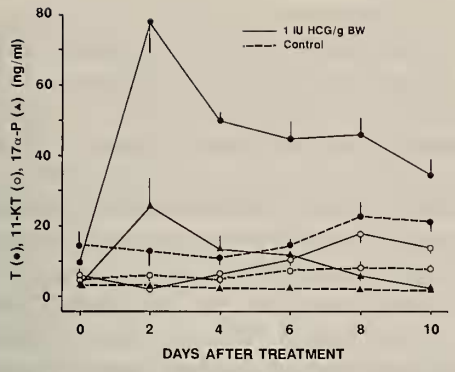


FIG. 8. Plasma testosterone, 11-KT, and 17$\alpha$-P levels following HCG injection in long-term experiment. Each point represented as mean $\pm$ SEM (n=8 and 6 for HCG and control group, respectively). Solid line is HCG treatment, dotted line is control.

to 1.8 ng/ml on day 2 ($P<0.01$; day 0 *vs* day 2). Thereafter levels increased, peaking on day 8 (17.6 ng/ml; $P<0.01$; day 2 *vs* day 8) and decreasing on day 10. No significant changes occurred in the control group.

Plasma 17$\alpha$-P levels peaked on day 2 (25.4 ng/ml; $P<0.01$; day 0 *vs* day 2), and then decreased to initial levels on day 10 (2.2 ng/ml). No significant changes occurred in the control group.

Changes in plasma 20$\beta$-S, 17$\alpha$,20$\alpha$-P, and 17$\alpha$,20$\beta$-P levels are presented in Figure 9. Plasma 20$\beta$-S levels started to increase on day 2 (7.4 ng/ml), peaking on day 4 (9.1 ng/ml; $P<0.01$; day 0 *vs* day 4) and returning to initial levels on day 10.

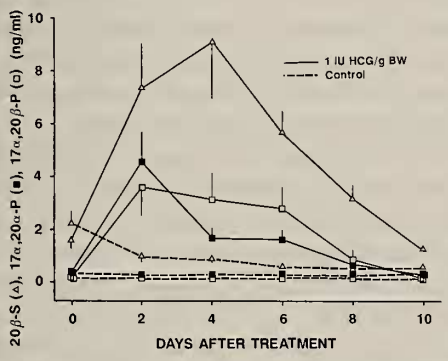


FIG. 9. Plasma 2$\beta$-S, 17$\alpha$,20$\alpha$-P, and 17$\alpha$,20$\beta$-P levels following HCG injection in long-term experiment. Each point represented as mean $\pm$ SEM (n=8 and 6 for HCG and control group, respectively). Solid line is HCG treatment, dotted line is control.

Meanwhile, levels in the control group decreased gradually ($P<0.01$; day 0 *vs* day 10).

Plasma 17$\alpha$,20$\alpha$-P levels in the HCG-treated group peaked on day 2 (4.5 ng/ml; $P<0.01$; day 0 *vs* day 2) and then returned to basal levels on day 10. No significant changes were observed in the control group.

Plasma 17$\alpha$,20$\beta$-P levels in the HCG-treated group also peaked on day 2 (3.8 ng/ml; $P<0.01$; day 0 *vs* day 2), and remained high until day 6, returning to the initial levels on day 10. No significant changes occurred in the control group.

Changes in GSI values following HCG injection in the long-term experiment are presented in Figure 7b. HCG injection caused significant increase in GSI from 0.14% to 0.18%.

Summary of the changes in levels of the six plasma steroids following HCG injection, both in short-term and long-term experiments are presented in Figure 10. 1) HCG firstly induces an increase in plasma testosterone and 11-KT levels. 2) Thereafter, 11-KT levels decrease and progestin levels increase. 3) Finally, progestin levels decrease, and 11-KT levels increase again.

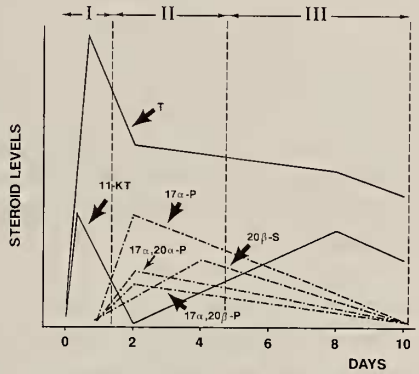


FIG. 10. Changes in levels of the six plasma steroids following HCG injection shown schematically for both the long- and short-term experiments. Lines represent trends, not absolute values. I: Androgen increase; II: Androgen decrease and progestin increase; and III: Progestin decrease and 11-ketotestosterone increase.

## DISCUSSION

A single injection of HCG in mature male tropical walking catfish caused significant changes

in all of the steroids monitored. HCG injection also caused enlargement of the testes.

HCG injection in both the 0.2 and 1 IU HCG-treated groups caused rapid increases in testosterone levels peaking at 18 hr. Thereafter, levels decreased but remained high. This suggests that the enzymes which are involved in testosterone synthesis are activated by HCG injection. In rainbow trout, gonadotropin has been suggested to stimulate the activity of two enzymes: $C_{17-20}$ lyase which converts 17$\alpha$-P to androstenedione, and 17$\beta$-HSD which converts androstenedione to testosterone [11, 15].

The function of testosterone in teleosts is still unclear, but is present in appreciable amounts during maturation in both males and females. Testosterone is regarded as an intermediate androgen product in the biosynthesis of 11-KT. However, based on replacement therapy in hypophysectomized catfish [17], it has been found that testosterone is able to maintain all stages of spermatogenesis, except spermatogonia mitosis. In addition, it has been shown also that testosterone isobutyrate crystals induce *in vitro* completion of spermatogenesis in undeveloped testes of goldfish [18]. In the present experiment, HCG injection elevated levels of testosterone which were much higher than those of 11-KT. This is in contrast to eel, in which the testosterone peak was lower than that of 11-KT [16]. These results suggest that the function of testosterone in male walking catfish is not merely to serve as a precursor to 11-KT synthesis.

The fluctuation pattern of 11-KT following HCG-injection in the present experiment is particularly interesting, since it showed two peaks. 11-KT levels peaked at 6 hr, decreased to initial levels at 36 hr, and then peaked again on day 8. The first peak was perhaps induced by the stimulatory effect of HCG on the conversion from testosterone to 11-KT, which is also observed in other fishes. The decreases, as will be discussed later, may be caused by the inhibitory effect of the progestin, as the decrease coincided with the increase in progestin levels. The fact that second peak occurred when progestin levels had already decreased, and that its magnitude was smaller than that of the first peak, suggests that the increase in the second peak was probably due to the elimination of the inhibitory effects of progestin. On the other hand, injection of HCG in dab [8] and goldfish [14], or salmon gonadotropin in brown bullhead [19] did not induce increases in 11-KT. One possible explanation is that these fish are annual spawners in which the sensitivity of testes to HCG or gonadotropin varies with time. However, under our stocking conditions, since plasma testosterone and 11-KT fluctuated at high levels throughout the year [26], male tropical walking catfish may possesses all developmental stages of the testes, so that fish can readily respond to HCG injection.

Plasma 17$\alpha$-P levels increased slowly after HCG injection and peaked at 72 hr, then decreased and returned to initial levels on day 10. This indicates that HCG treatment stimulates the testes to produce 17$\alpha$-P. Increased 17$\alpha$-P is converted into androstenedione by $C_{17-20}$ lyase, and testosterone production is increased as a result. However, it is considered that when lyase activity has been used to its maximum capacity, the conversion to progestin formation occurs, resulting the decrease in androgen production and the increase in 17$\alpha$,20$\alpha$-P, 17$\alpha$,20$\beta$-P, and 20$\beta$-S. This is in contrast to dab [8] in which HCG did not increase 17$\alpha$-P levels.

One of the interesting findings in the present experiment is the simultaneous increase in the levels of three progestins, 20$\beta$-S, 17$\alpha$,20$\alpha$-P and 17$\alpha$,20$\beta$-P, following HCG injection. Levels of 20$\beta$-S were highest, followed by 17$\alpha$,20$\beta$-P and 17$\alpha$,20$\alpha$-P. The occurrence of more than one progestin for example, 17$\alpha$,20$\alpha$-P and 3$\beta$,17,20$\alpha$-P-5$\beta$ in dab [8], and 17$\alpha$,20$\alpha$-P and 17$\alpha$,20$\beta$-P in carp [4] during spermiation in males has been reported. Progestin types seem to vary with species.

Little is known about the function of 20$\beta$-S in male fish. Our results show that HCG injection can elevate plasma 20$\beta$-S levels. Levels of this progestin were higher than those of 17$\alpha$,20$\alpha$-P, and 17$\alpha$,20$\beta$-P. The role of this hormone in the reproductive system of male walking catfish is still unknown. In HCG-injected female walking catfish as well, this hormone increases prior to ovulation [25].

17$\alpha$,20$\alpha$-P level increased due to HCG injection, and peaked at the day 2. High levels were main-

tained until the day 8. This increase suggests the presence of 20α-HSD in the male walking catfish. In the other teleosts, activity of this enzyme has been detected in spermatozoa of carp [2], dab and plaice [7]. The real function of this hormone in male walking catfish is yet unknown. In carp, however, this hormone has been suggested to be a spermiation regulator [2] as this hormone possessed an inhibitory effect on androgen production as did its isomer [3]. The fact that 17α,20α-P is produced at high levels by mature spermatozoa suggest that it is released into the environment at the time of spawning, and could therefore play an important behavioral/pheromonal role [2].

In this investigation, HCG injection possibly enhanced the activity of 20β-HSD which converts 17α-P to 17α,20β-P production. In the short-term experiment, 17α,20β-P levels started to increase after HCG injection, peaking at 72 hr. However, in the long-term experiment, this hormone peaked on day 2. Several roles have been proposed for 17α,20β-P. Based on an *in vitro* study in carp, it has been suggested that this hormone inhibits androgen production [4]. The hormone was reported to induced spermiation in amago salmon, *Oncorhynchus rhodurus* [24], and change the $K^+$/$Na^+$ ratio of rainbow trout seminal fluid [20]. Furthermore, pheromonal effects of this hormone have been reported to occur in the goldfish, *Carassius auratus* [21]. Recently, 17α,20β-P has been suggested to be a steroidal mediator in the spermiation of eel [16].

In the present experiment, HCG injection caused long-term changes in elevation of levels in all of the steroids monitored. These results were in contrast to those corresponding to females of the same species, in which HCG effects lasted only 24 hr [25]. The duration of steroid elevation following HCG injection in the present experiment is longer than the reported value during natural spawning or GtH treatment in other fishes. These differences may be due to the high dose and/or slow clearance of HCG in the male walking catfish. In goldfish, small doses of GtH (0.2 pg/g body weight) are cleared within 1 hr [10]. This has also been shown in *Ictalurus nebulosus* [19] injected with sGtH 5 to 500 ng/g body weight. The reason may be that the dosage used here is relatively high and that HCG is not an endogenous GtH.

In the short-term experiment, androgen levels were higher in the low dose group, whereas progestin levels were higher in high dose group. Lower levels in androgen and higher levels in progestin in the high dose group were probably caused by the conversion shift from androgen to progestin, since it is reported that high dose of gonadotropin induces the shift in salmonid [15]. Inhibition of androgen synthesis by increased progestin, which was found in carp [3], may also decrease androgen levels in catfish.

In summary, HCG injection increases testosterone production which is further converted to 11-KT. As a result, 11-KT production increases several hours after injection. Meanwhile, steroidogenic pathways favoring progestin production gradually become active. Increased progestin production possesses an inhibitory effect on 11-KT production. At this point, GSI increases and probably more sperm is produced. Consequently, 11-KT production decreases during progestin peaks, and increases again when progestin levels decrease.

## ACKNOWLEDGMENTS

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## REFERENCES

1 Aida K, Kato T, Awaji M (1984) Effects of castration on the smoltification of precocious male masu salmon *Oncorhynchus masou*. Bull Japan Soc Sci Fish 50: 565–571

2 Asahina K, Barry TP, Aida K, Fusetani N, Hanyu I (1990) Biosynthesis of 17α,20α-dihydroxy-4-pregnen-3-one from 17α-hydroxyprogesterone by spermatozoa of the common carp, *Cyprinus carpio*. J Exp Zool 255: 244–249

3 Barry TP, Aida K, Hanyu I (1989) Effect of 17α,20β-dihydroxy-4-pregnen-3-one on the *in vitro* production of 11-ketotestosterone by testicular fragments of the common carp, *Cyprinus carpio*. J Exp Zool 251: 117–120

4 Barry TP, Aida K, Okumura T, Hanyu I (1990) The shift from C-19 and C-21 steroid synthesis in spawning male common carp, *Cyprinus carpio*, is regulated by the inhibition of androgen production by progestogen produced by spermatozoa. Biol Reprod 43: 105–112

5 Barry TP, Santos AJG, Furukawa K, Aida K, Hanyu I (1990) Steroids profiles during spawning in male common carp. Gen Comp Endocrinol 80: 223–231

6 Baynes SM, Scott AP (1985) Seasonal variations in parameters of milt production and in plasma concentration of sex steroids of male rainbow trout (*Salmo gairdneri*). Gen Comp Endocrinol 57: 150–160

7 Canario AVM, Scott AP (1989) Synthesis of 20$\alpha$-hydroxylated steroids by ovaries of the dab (*Limanda limanda*). Gen Comp Endocrinol 76: 147–158

8 Canario AVM, Scott AP (1991) Levels of 17$\alpha$,20$\alpha$-dihydroxy-4-pregnen-3-one, and 3$\beta$,17$\alpha$,20$\alpha$-trihydroxy-5$\beta$-pregnane, and other sex steroids, in blood plasma of male dab, *Limanda limanda* (marine flatfish) injected with human chorionic gonadotropin. Gen Comp Endocrinol 83: 258–264

9 Colombo L, Belvedere PC, Simontacchi C, Lazzari M (1987) Shift from androgen to progesterone biosynthesis by the testes of the northern pike, *Esox lucius* L., during transition from spermatogenesis to spermiation. Gen Comp Endocrinol 66: 18 [Abstract 48]

10 Cook AF, Peter RE (1980) Plasma clearance of gonadotropin in goldfish, *Carassius auratus*, during the annual reproductive cycle. Gen Comp Endocrinol 42: 76–90

11 Depeche J, Sire O (1982) *In vitro* metabolism of progesterone in the testis of the rainbow trout, *Salmo gairdneri* Rich., at different stages of spermatogenesis. Reprod Nutr Develop 22: 427–438

12 Hunt SMV, Simpson TH, Wright RS (1982) Seasonal changes in the levels of 11-oxotestosterone and testosterone in the serum of male salmon, *Salmo salar* L., and their relationship to growth and maturation cycle. J Fish Biol 20: 105–119

13 Kobayashi M, Aida K, Hanyu I (1985) Radioimmunoassay for silvercarp gonadotropin. Bull Japan Soc Sci Fish 51: 1085–1091

14 Kobayashi M, Aida K, Hanyu I (1986) Effects of HCG on milt amount and plasma levels of steroid hormones in male goldfish. Bull Jap Soc Sci Fish 52: 755

15 Le Gac F, Loir M (1988) Control of testis function in fish: *in vitro* studies of gonadotropic regulation in the trout (*Salmo gairdneri*). Reprod Nutr Develop 28: 1031–1046

16 Miura T, Yamauchi K, Takahashi H, Nagahama Y (1991) Involvement of steroid hormones in gonadotropin-induced testicular maturation in male Japanese eel (*Anguilla japonica*). Biomed Res. 12: 241–248

17 Nayyar SK, Keshavanath P, Sundararaj BI, Donaldson EM (1976) Maintenance of spermatogenesis and seminal vesicles in hypophysectomized catfish, *Heteropneustes fossili* (Bloch): effects of ovine and salmon gonadotropin and testosterone. Can J Zool 54: 285–292

18 Remacle C (1976) Actions hormonales sur les cellules germinales males de *Carassius auratus* L. en culture oranotypique. Gen Comp Endocrinol 29: 480–491

19 Rosenblum PM, Callard IP (1987) Response of male brown bullhead catfish, *Ictalurus nebulosus* Lesueur, to gonadotropin-releasing hormone and gonadotropin. J Exp Zool 243: 189–199

20 Scott AP, Baynes SM (1982) Plasma levels of sex steroids in relation to ovulation and spermiation in rainbow trout (*Salmo gairdneri*). Proc Second Int Symp on the Reprod Physiol of Fish, Wageningen, The Netherlands, pp 103–106

21 Stacey NE, Sorensen PW, Dulka JG, Van der Kraak GJ, Hara TJ (1987) Teleost sex pheromone: Recent studies on identity and function. Proc Third Int Symp on the Reprod Physiol of Fish, St John's, Newfoundland, pp 150–153

22 Trant JM, Thomas P (1989a) Evidence that 17$\alpha$,20$\beta$,21-trihydroxy-4-pregnen-3-one is a maturation inducing steroid in spotted seatrout. Fish Physiol Biochem 7: 185–191

23 Trant JM, Thomas P (1989b) Isolation of a novel maturation-inducing steroid produced *in vitro* by ovaries of Atlantic croaker. Gen Comp Endocrinol 75: 397–404

24 Ueda H, Young G, Crim LW, Kambegawa A, and Nagahama Y (1983) 17$\alpha$,20$\beta$-dihydroxy-4-pregnen-3-one: Plasma levels during sexual maturation and *in vitro* production by the testes of amago salmon (*Oncorhynchus rhodurus*) and rainbow trout (*Salmo gairdneri*). Gen Comp Endocrinol 51: 106–112

25 Zairin M Jr; Asahina K, Furukawa K, Aida K (1992) Plasma steroid hormone profiles during HCG induced ovulation in female walking catfish *Clarias batrachus*. Zool Sci 9: 607–617

26 Zairin M Jr (1993) Endocrinological studies on maturation and spawning in the walking Catfish, *Clarias batrachus*. Doctoral thesis, The University of Tokyo, pp 1–90